CLAIMS

1. A process for producing recombinant albumin, the process comprising culturing a fungal cell expressing a recombinant albumin coding sequence and obtaining the albumin, wherein the cell has a genetic modification which causes the cell to have at least a reduced capacity of mannosylation of the recombinantly-expressed albumin and wherein the culture medium is at least 1,000L and is of pH5. 3-6.8

2. A process according to Claim 1 wherein said modification(s) comprises any suppression, substitution, deletion, addition, disruption and/or mutational insertion.

3. A process according to Claim 2 wherein said modification(s) are stably-inherited and/or are non-reverting and/or are non-leaky.

4. A process according to any one of Claims 1 to 3 wherein said modification(s) are located in a coding region of a gene or in a region involved in the expression of a gene.

5. A process according to claim 4 wherein the gene is a *PMT* gene, preferably *PMT1*.

6. A process according to any one of the preceding claims wherein the fungal cell is cultured in a culture medium of at least 5,000L, preferably at least 7,500L.

7. A process according to any one of the preceding claims wherein the fungal cell is cultured at pH6.2-6.7, preferably pH6.3-6.5.

8. A process for purifying an albumin solution, the process comprising the step of subjecting a first albumin solution of pH8.0-9.5, and having a conductivity in the range of 1 to 75mS.cm^{-1}, to an affinity chromatography step which is run in negative mode with respect to the albumin and which utilises an affinity matrix comprising immobilised dihydroxyboryl groups, thereby obtaining a purified albumin solution.

9. A process according to Claim 8 wherein the pH of the first albumin solution is pH8.0-9.0, preferably pH8.3-pH8.6.

10. A process according to Claim 8 or 9 wherein the first albumin solution is buffered with a buffer which comprises glycine at a concentration of 10-500mM, preferably 25-200mM, and more preferably 50-150mM; NaCl at a concentration of 0-500mM, preferably 25-200mM, and more preferably 50-150mM; and $CaCl_2$ at a concentration of 5-250mM, preferably 10-100mM.

11. A process according to Claim 10 wherein the first albumin solution is buffered with a buffer which comprises about 100mM glycine, about 100mM NaCl and about 50mM $CaCl_2$.

12. A process according to Claim 10 or 11 wherein the conductivity of the buffer is 10-50mS.cm^{-1}, preferably 18-22mS.cm^{-1}.

13. A process according to any one of Claims 8 to 12 wherein the first albumin solution comprises albumin at a concentration of at least 70g.L^{-1}.

14. A process according to any one of Claims 8 to 13 wherein the albumin is loaded in less than 0.5 column volumes, more preferably in less than 0.35 column volumes.

15. A process according to any one of Claims 8 to 14 wherein the matrix comprises immobilised boronic acid, preferably aminophenylboronic acid.

16. A process according to any one of Claims 8 to 15 wherein the purified albumin solution is subjected to further purification using cation exchange chromatography, to yield a cation exchange-purified albumin solution.

17. A process according to any one of Claims 8 to 16 wherein the purified albumin solution is subjected to further purification using anion exchange chromatography, to yield an anion exchange-purified albumin solution.

18. A process according to Claim 17 when dependent on any one of Claims 8 to 15 and wherein the anion exchange-purified albumin solution is subjected to further purification using cation exchange chromatography, to yield a cation exchange-purified albumin solution.

19. A process according to any one of Claims 8 to 18 wherein the purified albumin solution undergoes one or more of: buffer exchange; concentration; dilution; dialysis; diafiltration; pH-adjustment; treatment with a reducing agent; decolouration treatment; heating; cooling; or conditioning.

20. A process for purifying an albumin solution, the process comprising cation exchange chromatography followed by anion exchange chromatography, or anion exchange chromatography followed by cation exchange chromatography, wherein the albumin solution from the second said chromatographic step does not undergo further purification, prior to being put into a final container.

21. A process according to Claim 16, 18 or 20 wherein the cation exchange step is run in negative mode with respect to the albumin.

22. A process according to Claim 21 wherein glycosylated albumin binds to the cation exchange material.

23. A process according to Claim 16, 18, 20, 21 or 22 wherein the cation exchange step utilises a matrix which comprises immobilised sulfopropyl substituents as cation exchangers.

24. A process according to any preceding claim which claims specifies cation exchange chromatography wherein the albumin solution which undergoes cation exchange chromatography has a pH of 4.5-6.0, more preferably a pH of 5.0-5.6, and yet more preferably a pH of 5.2-5.4.

25. A process according to any preceding claim which claims specifies cation exchange chromatography wherein the albumin solution which undergoes cation exchange chromatography has an albumin concentration of 10-250g.L^{-1}, preferably 20-70g.L^{-1}, and more preferably 50±10g.L^{-1}.

26. A process according to any preceding claim which claims specifies cation exchange chromatography wherein the albumin solution which undergoes cation exchange chromatography has an octanoate ion concentration of 2-15mM, preferably 5-10mM, and more preferably 6-9mM.

27. A process according to any preceding claim which claims specifies cation exchange chromatography wherein prior to the cation exchange step the albumin solution undergoes one or more of the following processes: (i) pH-adjustment; (ii) concentration; (iii) diafiltration; or (iv) conditioning by addition of octanoate and/or other fatty acid.

28. A process according to Claim 17 or 20 wherein the anion exchange step utilises a matrix which comprises immobilised dialkylaminoalkyl substituents as anion exchangers.

29. A process according to any one of Claims 17, 20 or 28 wherein the anion exchange step is run in negative mode with respect to the albumin.

30. A process according to Claim 29 wherein the albumin solution which undergoes anion exchange chromatography has a pH of 4.0-5.2, more preferably a pH of 4.2-4.9, and yet more preferably a pH of 4.5-4.7.

31. A process according to Claim 29 wherein the albumin solution which undergoes anion exchange chromatography has a conductivity of less than 4.0mS.cm^{-1}, and preferably a conductivity of 1.0±0.5mS.cm^{-1}, and more preferably a conductivity of 1.05±0.1mS.cm^{-1}.

32. A process according to any one of Claims 17, 20 or 28 wherein the anion exchange step is run in positive mode with respect to the albumin.

33. A process according to Claim 32 wherein the albumin solution which undergoes positive mode anion exchange chromatography has a pH of 6.0-8.0, preferably a pH of 6.5-7.5, and yet more preferably a pH of 6.8-7.2.

34. A process according to Claim 32 or 33 wherein the concentration of the albumin in the albumin solution which undergoes positive mode anion exchange chromatography is 10-100g.L^{-1}, more preferably 25-70g.L^{-1}, and most preferably 30-50 g.L^{-1}.

35. A process according to any one of Claims 32, 33 or 34 wherein the albumin solution which undergoes positive mode anion exchange chromatography has a conductivity of 1.0-1.5mS.cm^{-1}, preferably 1.2-1.4mS.cm^{-1}.

36. A process according to Claim 32 or 33 wherein the concentration of the albumin in the albumin solution which undergoes positive mode anion exchange chromatography is 10-100g.L^{-1}, more preferably 25-80g.L^{-1}, and most preferably 40-60g.L^{-1}.

37. A process according to any one of Claims 32, 33 or 36 wherein the albumin solution which undergoes positive mode anion exchange chromatography has a conductivity of 1.0-2.0mS.cm^{-1}, preferably 1.2-1.6mS.cm^{-1}, and more preferably 1.3-1.5 mS.cm^{-1}.

38. A process according to any one of Claims 32-37 wherein the albumin is eluted from the anion exchanger using a buffer comprising a compound having a specific affinity for albumin, preferably an acid.

39. A process according to Claim 38 wherein the buffer comprises 20-90mM, preferably 30-70mM and more preferably 35-65mM of a phosphoric acid salt, preferably sodium phosphate.

40. A process according to Claim 38 or 39 wherein the albumin is eluted from the anion exchanger with a buffer of pH6.0-8.0, preferably pH6.5-7.5.

41. A process according to Claim 29 or 32 wherein, prior to the anion exchange step, the albumin solution undergoes one or more of: buffer exchange; concentration; dilution; dialysis; diafiltration; pH-adjustment; treatment with a reducing agent; decolouration treatment; heating; cooling or conditioning.

42. A process according to any one of Claims 8 to 41 which is preceded by one or more of the following steps: fermentation; primary separation; centrate conditioning; cation exchange chromatography, preferably using sulfopropyl substituents as cation exchangers; anion exchange chromatography, preferably using diethylaminoalkyl substituents as anion exchangers; or affinity chromatography, preferably using an affinity matrix which comprises an immobilised albumin-specific dye, preferably a Cibacron Blue type of dye.

43. A process for purifying an albumin solution, the process comprising the steps of:

(a) subjecting an albumin solution to a cation exchange chromatography step run in positive mode with respect to the albumin;

(b) collecting an albumin-containing cation exchange eluate;

(c) subjecting the cation exchange eluate to an anion exchange chromatography step run in positive mode with respect to the albumin;

(d) collecting an albumin-containing anion exchange eluate;

(e) subjecting the anion exchange eluate to an affinity chromatography step run in positive mode with respect to the albumin;

(f) collecting an albumin-containing affinity chromatography eluate;

(g) subjecting the affinity chromatography eluate to an affinity chromatography step run in negative mode with respect to the albumin and in positive mode with respect to glycoconjugates;

(h) collecting the albumin-containing affinity chromatography flow through;

(i) subjecting the affinity chromatography flow through to a cation exchange chromatography step run in negative mode with respect to the albumin;

(j) collecting the albumin-containing cation exchange flow through;

(k) subjecting the cation exchange flow through to an anion exchange chromatography step run in negative mode or positive mode;

(l) collecting the albumin-containing anion exchange flow through wherein the anion exchange step is run in negative mode; or eluting from the anion exchange matrix an anion exchange eluate wherein the anion exchange step is run in positive mode;

and wherein any of the respective purification steps are optionally preceded or followed by one or more of: buffer exchange; concentration; dilution; dialysis; diafiltration; pH-adjustment; treatment with a reducing agent; decolouration treatment; heating; cooling or conditioning.

44. A process for purifying an albumin solution, the process comprising the steps of:

(a) subjecting an albumin solution to a cation exchange chromatography step run in positive mode with respect to the albumin;

(b) collecting an albumin-containing cation exchange eluate;

(c) subjecting the cation exchange eluate to an anion exchange chromatography step run in positive mode with respect to the albumin;

(d) collecting an albumin-containing anion exchange eluate;

(e) subjecting the anion exchange eluate to an affinity chromatography step run in positive mode with respect to the albumin;

(f) collecting an albumin-containing affinity chromatography eluate;

(g) subjecting the affinity chromatography eluate to an affinity chromatography step run in negative mode with respect to the albumin and in positive mode with respect to glycoconjugates;

(h) collecting the albumin-containing affinity chromatography flow through;

(i) subjecting the affinity matrix flow through to an anion exchange chromatography step run in negative or positive mode with respect to the albumin;

(j) collecting the albumin-containing anion exchange flow through wherein the anion exchange step is run in negative mode; or eluting from the anion exchange matrix an anion exchange eluate wherein the anion exchange step is run in positive mode;

(k) subjecting the albumin solution purified by the anion exchange chromatography step to a cation exchange chromatography step run in negative mode with respect to the albumin;

(l) collecting the albumin-containing cation exchange flow through;

and wherein any of the respective purification steps are optionally preceded or followed by one or more of: buffer exchange; concentration; dilution; dialysis; diafiltration; pH-adjustment; addition of reducing agent; decolouration treatment; heating; cooling or conditioning.

45. A process for reducing the level of nickel ions in an albumin solution, the process comprising subjecting an albumin solution to a pH of 2.5-7.5, preferably pH4.0 to pH6.0, and removing nickel ions.

46. A process according to Claim 45 wherein the process comprises diafiltration or gel permeation chromatography.

47. A process according to any one of Claims 8 to 46 wherein the albumin is recombinant albumin.

48. A process according to any one of Claims 8 to 47 wherein the initial albumin solution is derived from a yeast culture medium obtained by culturing yeast transformed with an albumin-encoding nucleotide sequence in a fermentation medium, whereby said yeast expresses albumin and secretes it into the medium.

49. A process according to Claim 48 wherein the yeast is of the genus *Saccharomyces,* preferably *Saccharomyces cerevisiae*.

50. A process according to Claim 48 wherein the yeast is of the genus *Pichia* or *Kluyveromyces*.

51. A process according to any one of Claims 8 to 50, wherein at least some of the albumin is produced by a process according to any one of Claims 1 to 7 or by a cell according to Claim 53.

52. A process according to any one of Claims 1-51 wherein the albumin product is formulated for parenteral administration to a human, sterilised or placed into a final container.

53. A DNA sequence, plasmid or cell which comprises a recombinant albumin coding sequence wherein the 3′ end of the recombinant albumin coding sequence comprises two or more ~~in-frame~~ translation stop codons, and preferably three in-frame translation stop codons.

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